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MORPHOLOGICAL AND HOST-SYMBIONT STUDIES OF *TRICHODINA TENUIFORMIS* AND *APIOSOMA CAMPANULATUM* INFESTING MOTTLED SCULPIN (*COTTUS BAIRDI*) FROM PROVO RIVER, UTAH

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Abstract.—*Trichodina tenuiformis* Stein, 1979 and *Apiosoma campanulatum* Timofeev, 1962 were found on gills of mottled sculpin (*Cottus bairdi*) from two locations in the Provo River, UT. They were studied by light and electron optics. Dimensions and morphology of the adhesive disc and denticles of *T. tenuiformis* were differentiated from other *Trichodina* species. *A. campanulatum* was characterized by its spindle-shaped cell body. Fine features examined by scanning electron microscopy included body shape, pellicle, elements of the adhesive disc, aboral ciliary complex, and adoral ciliary spiral. Histopathological studies suggested that the organisms are ectocommensals. Ecological aspects of organism infestation between two areas were also investigated. This report establishes a new host and distribution record for these two species in mottled sculpin from the Provo River, UT.

Key words: Trichodina tenuiformis, Apiosoma campanulatum, Cottus bairdi, *morphology, host-symbiont relationship, ecological aspects, Provo River.*

High numbers of two ciliated protozoa, *Trichodina* and *Apiosoma*, were encountered on the gills of mottled sculpin (*Cottus bairdi*) during a study of ectoparasites of fishes from the Provo River.

Trichodina is a mobile ciliate belonging to the subclass Peritrichia, family Trichodinidae (Lom and Dykova 1992). This protozoan has an adhesive disc characterized by very prominent and taxonomically significant denticles (Van As and Basson 1987). More than 140 species of *Trichodina* have been reported from wild, cultured, and laboratory fishes in many parts of the world (Rand 1993).

Sessile peritrich ciliates of the genus *Apiosoma* (syn. *Glossatella*) belong to the subclass Peritrichia, family Epistylididae (Lom and Dykova 1992). They are generally attached to fish by a scopula (Lom 1973). They have been largely neglected by fish parasitologists until recently, when more attention has been given to this group.

Many species of these two ciliated protozoa have been investigated (Arthur and Margolis 1984, Cone and Odense 1987, Rand 1993); however, a detailed study on mottled sculpin has never been reported. Objectives of this study were to (1) incorporate different levels of microscopy to study ciliate structure, (2) observe histopathological changes these protozoa may cause to the host, and (3) evaluate the seasonal infestation rate to provide ecological information for the listed ciliates and their host.

Materials and Methods

Studies were carried out in late summer and fall (August, October 1993), late winter and spring (March, May 1994). Water temperatures in the Provo River ranged from 14°C to 4°C and 6°C to 10°C, respectively. One hundred sixty sculpin were collected from two sites: one in the city of Provo (Utah County) municipal area, the second in a relatively pristine region near the Jordanelle Reservoir (Wasatch County). Sculpin were collected using electrofishing, placed in buckets containing river water, transported to the laboratory, and examined within 24 h after capture.

For light microscopy, air-dried smears of gill filament scrapings were prepared from freshly killed fish and treated by Klein's dry silver impregnation technique (Clark and Heckmann 1984) to examine components of the adhesive disc. Other smears were prepared, fixed, air-dried, and stained with iron hematoxylin (Garcia and Bruckner 1988) to observe the position and structure of the macro- and micronuclei. Sections of infested gills from the spring sample were fixed, blocked, cut, and

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stained with hematoxylin-eosin (Garcia and Bruckner 1988) for histopathological studies.

For scanning electron microscopy, gills of freshly killed fish were fixed in 2% buffered glutaraldehyde, followed by repeated washes in a sodium cacodylate buffer and post-fixed in a 1% solution of osmium tetroxide. After that they were washed in the same buffer system. Specimens were dehydrated through a graded alcohol series and critical-point-dried and sputter-coated with gold for examination with a Joel-840 high-resolution scanning electron microscope.

For transmission electron microscopy, after fixation and dehydration, gills were embedded in Spurr resin and sectioned with a glass knife. Each section was stained with lead citrate and examined with a Philip EM400 transmission electron microscope.

Terminology and methods of measurement follow those given by Lom (1958), Lom and Dykova (1992), Wellborn (1967), Arthur and Margolis (1984). Measurements are in micrometers (μm) and are based on 30 specimens for each species from each of the four sampling periods; range is followed by the mean and ± standard deviation in parentheses.

RESULTS

Morphology

Trichodina tenuiformis Stein, 1979

HOST.—*Cottus bairdi* (Pisces: Cottidae).

LOCALITY.—Provo River, Utah and Wasatch counties, Utah.

SITE OF INFESTATION.—Gill filaments.

LIGHT MICROSCOPY.—Body 39–53 (44.2 ± 4.0) dia (diameter). Adhesive disc 19–30 (26.3 ± 2.8) dia, surrounded by a border membrane 2–3 (2.5 ± 0.4) wide, with fine transverse striae. Various-sized light forms present in center of adhesive disc when silver-impregnated. Denticular ring 13.5–20 (17.2 ± 1.8) dia, consisting of 20–26 (23.7 ± 1.3) denticles with 6–10 (7.8 ± 0.8) radial pins per denticle. Denticle with conical central portions 0.7–1 (0.99 ± 0.06) from which a thorn 2.5–4 (2.9 ± 0.4) extends externally with broadly rounded lobes, tapered slightly to a blunt tip and blade 2–3 (2.3 ± 0.3) attached to central region, some with rounded ends (Figs. 1, 2).

Macronucleus horseshoe-shaped 27–48 (39 ± 5.7) dia and approximately 10 μm thick. Micronucleus in -Y position (Lom 1958) observed in six specimens, dimension 3 × 2 (Fig. 3).

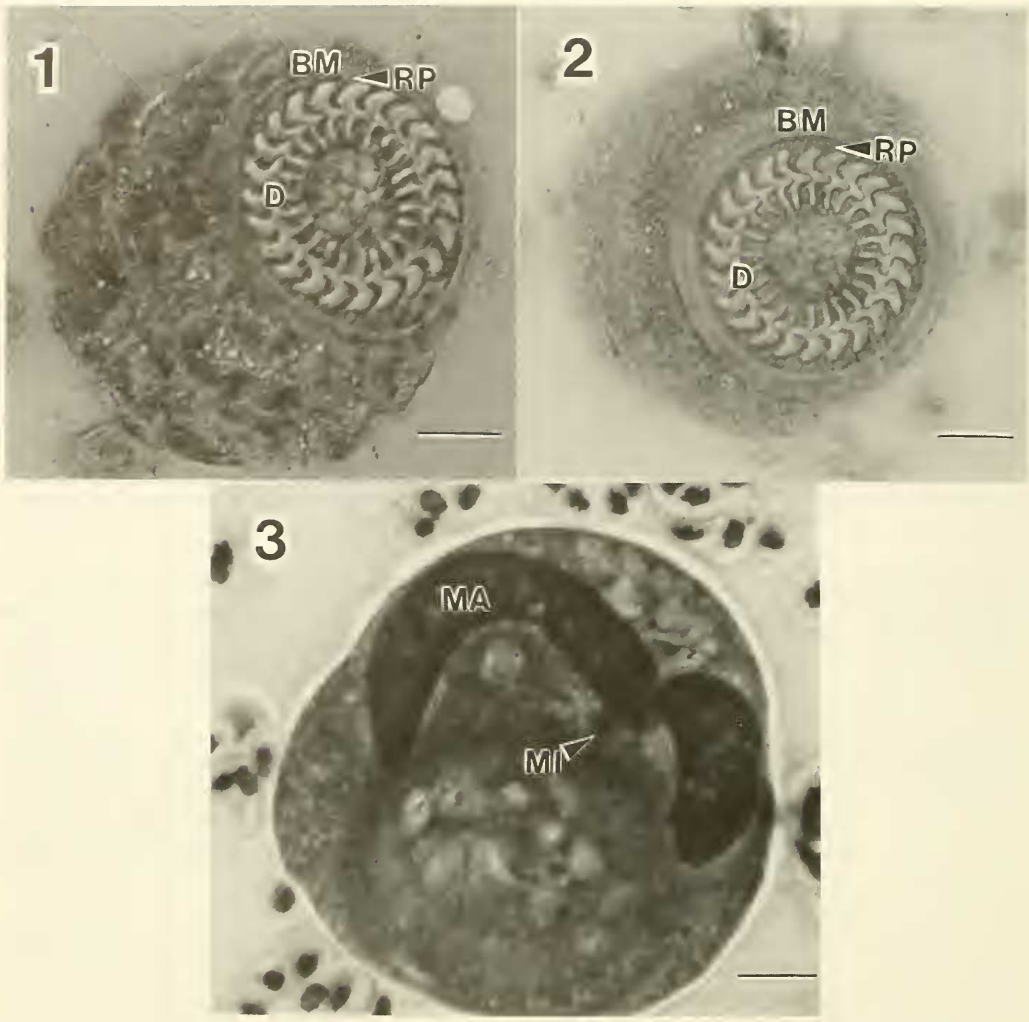
SCANNING ELECTRON MICROSCOPY.—Body of *T. tenuiformis* circular in aboral view and aboral surface relatively flat (Fig. 4). Body bell-shaped or domed when viewed from the side (Fig. 5).

The aboral ciliary complex consists of three distinct ciliary bands: the basal ciliary ring, locomotor ciliary wreath, and marginal ciliary ring. The basal ciliary ring, adjacent to the border membrane, has a single row of fine, distally tapering cilia 1–2 μm long. Separated from the basal ciliary ring by the basal septum is the locomotor ciliary wreath, which is composed of numerous rows of well-developed, powerful cilia 2–3 μm long whose primary function is locomotion. The precise number of ciliary rows composing this wreath could not be ascertained. It is separated anteriorly from the marginal ciliary ring by a poorly developed anterior septum that is evident only when the aboral ciliary complex is uncovered by the velum. The marginal ciliary ring is difficult to distinguish from the locomotor ciliary wreath in *T. tenuiformis*. The velum is a thick, well-developed structure covering the bases of the cilia of the aboral ciliary complex and separating this complex from the adoral ciliary spiral (Figs. 5, 6).

The adhesive disc has a smooth pellicular surface beneath which the outline of the denticles can be clearly seen. The disc is surrounded peripherally by a 2-μm-wide border membrane, which functions to seal the margin of the disc during adherence and contains fine vertical striae over its entire surface. These striae on the internal surface of the border membrane are the radial pins that give the membrane rigidity while retaining its ability to conform to the host's surface (Fig. 7).

The adoral ciliature forms a counterclockwise spiral of about 270°. The base of each cilium is inserted into a deep furrow and hidden from view when SEM is used (Fig. 8).

DEPOSITION OF SLIDES.—One slide (HWML 37721) of silver-impregnated specimens and another slide (HWML 37724) of iron-hematoxylin–stained specimens are deposited in the Harold W. Manter Laboratory, University of Nebraska State Museum. The senior author has additional slides in her collection.



Figs. 1–3. Light micrographs of *Trichodina tenuiformis:* 1–2. Silver-impregnated specimens showing body shape and arrangement of components of the adhesive disc. BM, border membrane; D, denticle; RP, radial pins. Bar = 10 μm. 3. Iron-hematoxylin–stained specimen showing horseshoe-shaped macronucleus (MA); arrow points to the micronucleus (MI). Bar = 10 μm.

Apiosoma campanulatum Timofeev, 1962

HOST.—*Cottus bairdi* (Pisces: Cottidae).

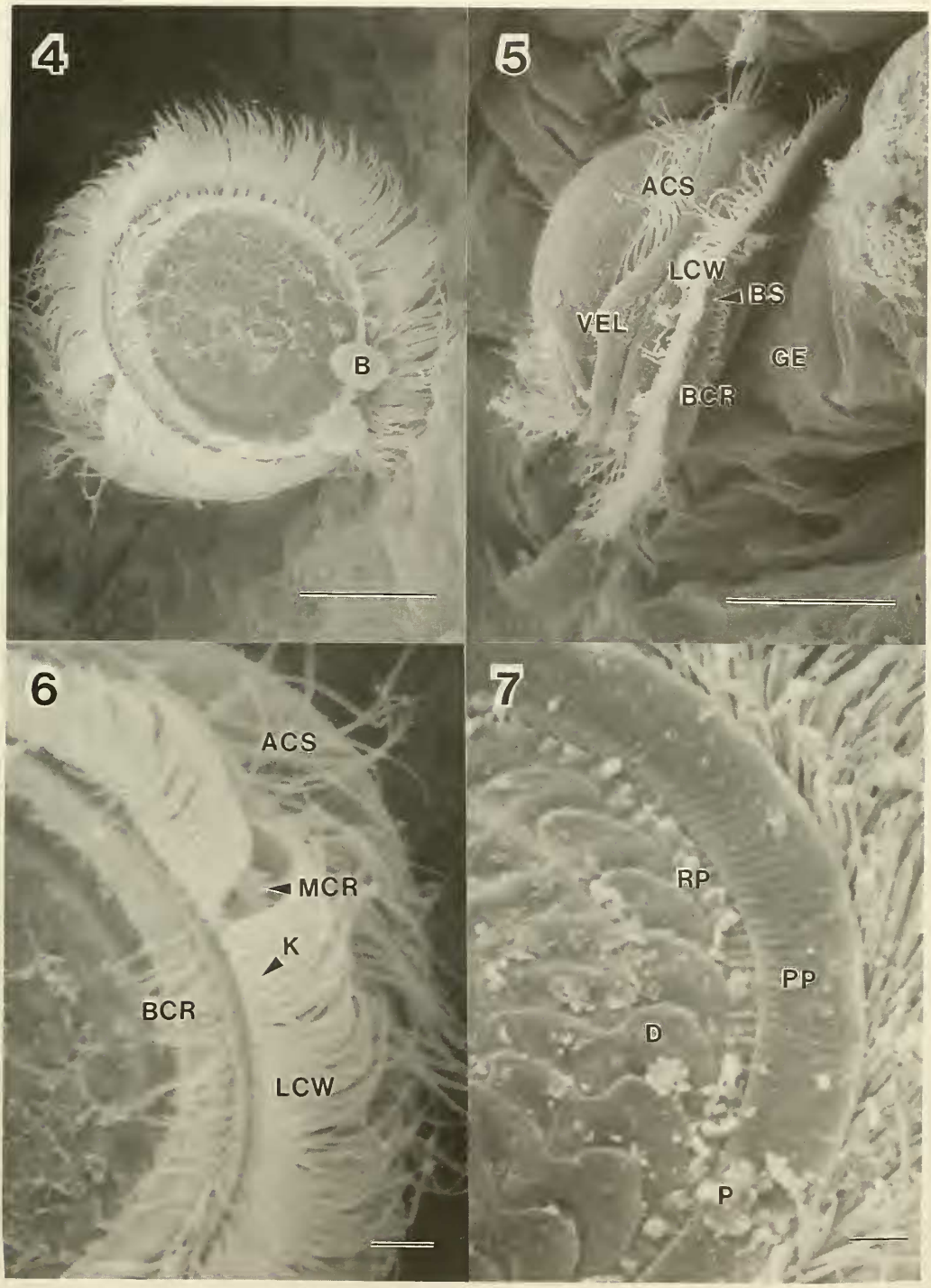
LOCALITY.—Provo River, Utah and Wasatch counties, UT.

SITE OF INFESTATION.— Gill filaments.

LIGHT MICROSCOPY.—Body campanulate. Macronucleus round or slightly conical. Size of stained specimens 31.0–66.0 (47.8 ± 7.2) long by 25.0–45.0 (35.6 ± 4.2) wide. Macronucleus 11.0–20.0 (15.6 ± 2.4). Micronucleus not observed (Fig. 9).

SCANNING ELECTRON MICROSCOPY.—The spindle-shaped body is the characteristic feature of this species. Circular striations of pellicle conspicuous. Pellicle wrinkled into longitudinal furrows. Upper part of body bears the adoral zone, consisting of a tuft of 1–2-μm-long cilia. Most specimens viewed with SEM have contracted peristomes and contracted peristomial lips (Fig. 10).

DEPOSITION OF SLIDES.—A representative slide of *Apiosoma campanulatum* (silver stain) is deposited in the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML 37722). The senior author has additional slides in her collection.



Figs. 4–7. Scanning electron micrographs of the surface of *T. tenuiformis:* 4. Aboral view of entire specimen of *T. tenuiformis.* B, bacteria. Bar = 10 μm. 5. Lateral view of entire specimen of *T. tenuiformis.* ACS, adoral ciliary spiral; BS, basal septum; BCR, basil ciliary ring; GE, gill epithelium; LCW, locomotor ciliary wreath; VEL, velum. Bar = 10 μm. 6. Higher magnification of Figure 4 showing the structure of aboral ciliary complex. ACS, adoral ciliary spiral; BCR, basal ciliary ring; K, kinetosomes; LCW, locomotor ciliary wreath; MCR, marginal ciliary ring. Bar = 1 μm. 7. Adhesive disc of *T. tenuiformis.* D, denticle; PP, peripheral pins; RP, radial pins. Bar = 1 μm.

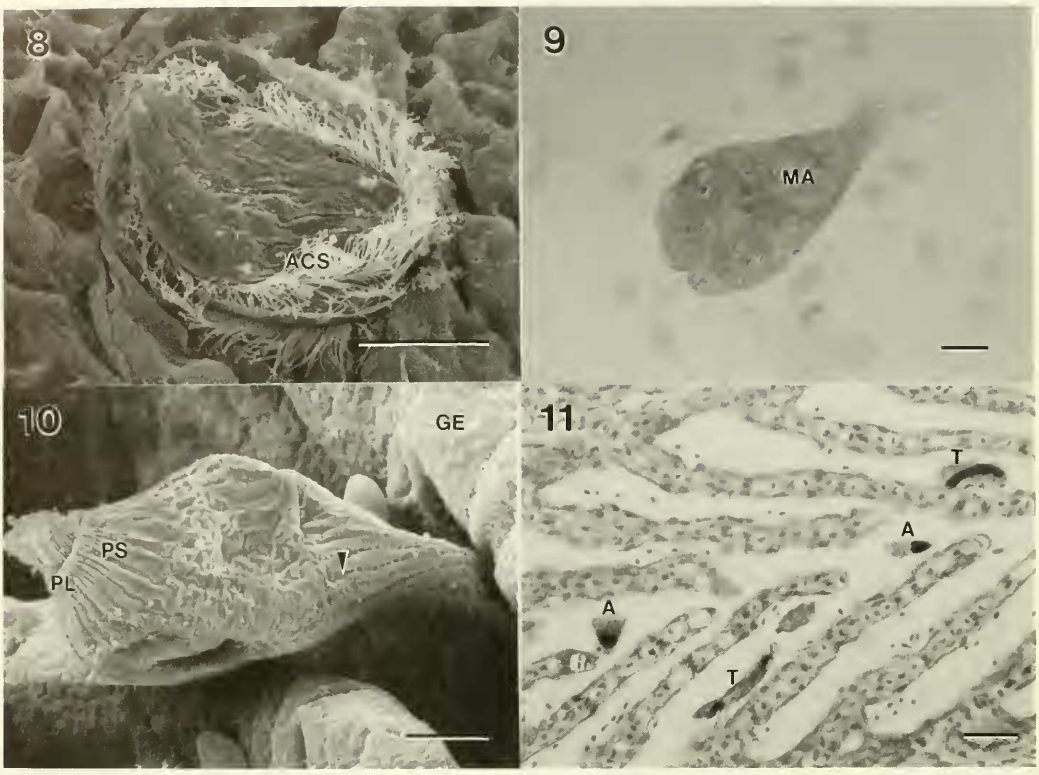


Fig. 8. Adoral view of *T. tenuiformis* showing how the adoral ciliature (ACS) forms a counterclockwise spiral of about 270°. Bar = 10 μm. Fig. 9. Light micrograph of *Apiosoma campanulatum*. Note conicle-shaped body. MA, macronucleus. Bar = 1 μm. Fig. 10. Scanning electron micrograph of *A. campanulatum* attached to the gill epithelium (GE). Note transverse striations of pellicle and its longitudinal furrows (arrow). PL, peristomal lip; PS, peristome. Bar = 5 μm. Fig. 11. Light micrographs showing *Trichodina* (T) and *Apiosoma* (A) infested gill epithelium. Bar = 20 μm.

Host-Symbiont Relationships

LIGHT MICROSCOPY.—Sections of mottled sculpin gills had no apparent pathological damage. The conical body of some *A. campanulatum* appeared to be attached to host gill surfaces by the scopula, while others were freely distributed over the epithelial surface. Most *T. tenuiformis* glide over the surface; only a few ciliates adhere to the host epithelial cells (Fig. 11).

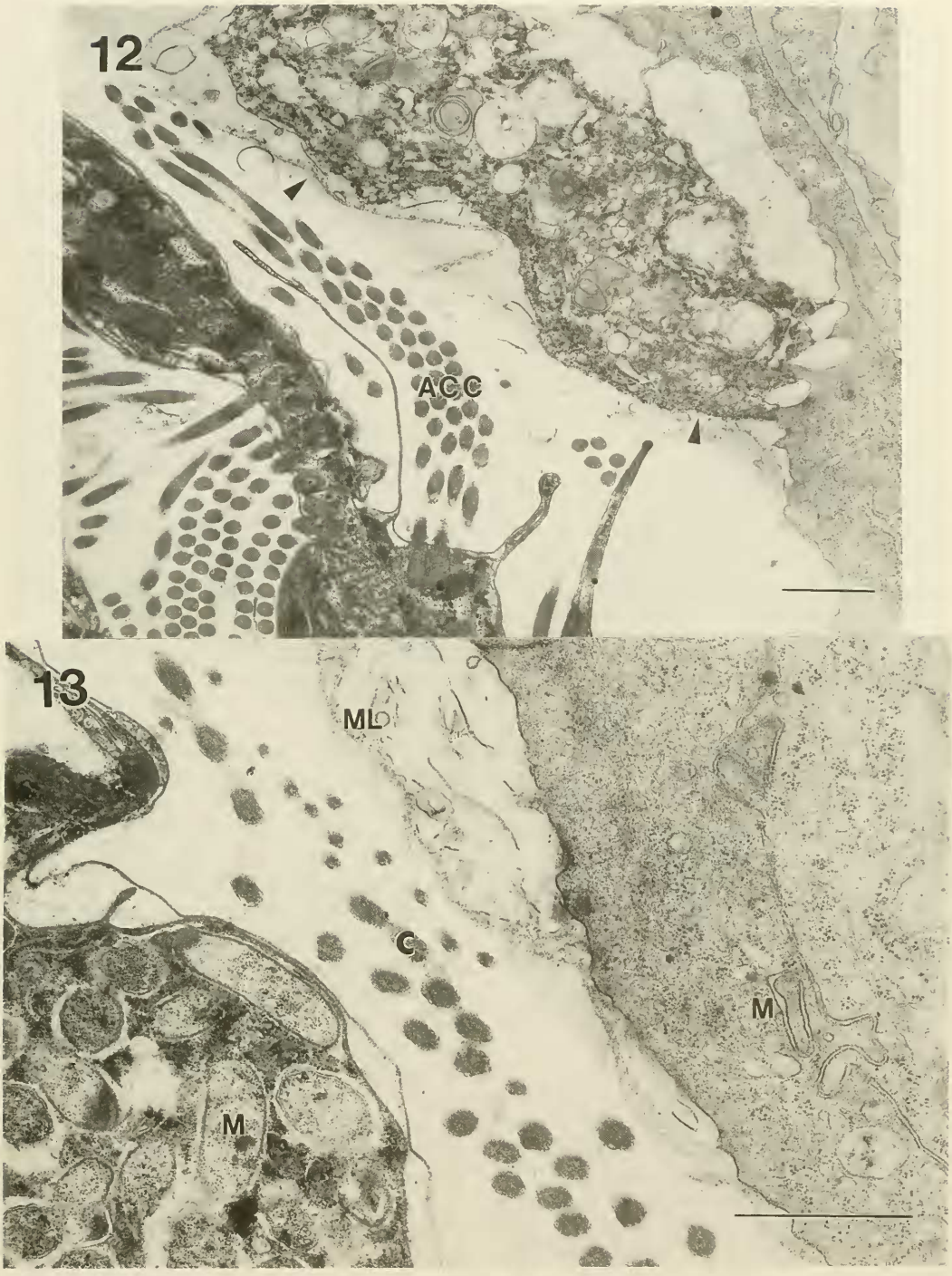
TRANSMISSION ELECTRON MICROSCOPY.—Sections of the interface between the host epithelial cell and *T. tenuiformis* were prepared. No permanent or temporary structure could be detected between the adhesive disc, adoral zone of cilia, and gill epithelial cells (Fig. 12). However, injury to the epithelium due to *T. tenuiformis* can be detected by the number of mitochondria, which decrease and disappear in the immediate host cell. Host necrotic tissue, mucous layers from gill epithelium, and particles dispersed in the water were on the surface of *T. tenuiformis* (Fig. 13).

No ultrastructural damage was observed for *A. campanulatum*. Presence of this ciliate inflicts no serious damage to the host cell. There was some change in number of mitochondria, with cristae showing major changes (Fig. 14).

Ecological Aspects of Infestation

In the Provo River near the Provo residential area, *T. tenuiformis* reached the highest infestation rate in April and May. It was uncommon during summer and autumn and appeared to be absent in the winter. With the increase of water temperature in spring, ciliates reinfested the fish. *Apiosoma campanulatum* at this site maintained an average of 35% infestation rate (no. of infested fish vs. no. of total examined fish) for all seasons.

In the upper Provo River the tendency of infestation of *T. tenuiformis* corresponded closely



Figs. 12–13. Transmission electron micrographs of gill epithelium infested by *T. tenuiformis*. 12. Host necrotic tissue (arrows) sloughs off for parasite's food. ACC, aboral ciliary complex. Bar = 1 μm. 13. Interface between *T. tenuiformis* and mucous layer (ML) of epithelial cells. Note damage to mitochondria (M). C, cilia. Bar = 1 μm.

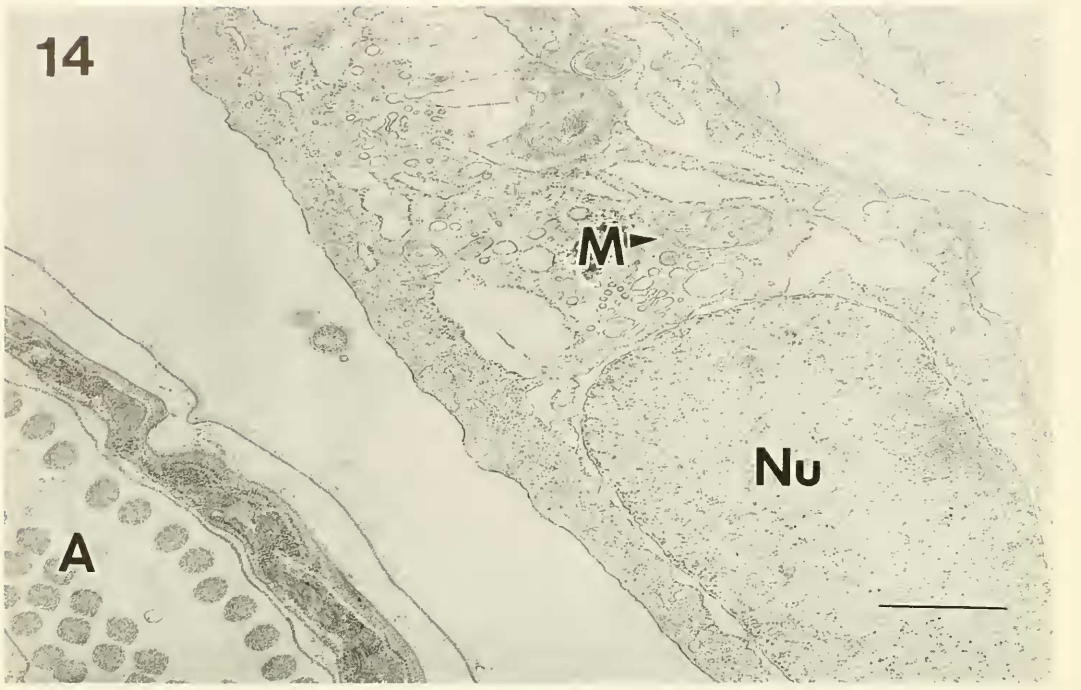


Fig. 14. Transmission electron micrograph of gill epithelium infested by *A. campanulatum*. *A. campanulatum* (A) causes number of mitochondria (M) to decrease and cristae to disappear. M, mitonchondria; Nu, nucleus of epithelial cell. Bar = 1 μm.

to that of the lower area. The highest infestation rate occurred in May and then decreased until the next spring. Percentage of fish infested by *T. tenuiformis* in the lower river area was 20.5% vs. 12.5% in the upper Provo River. Similar to that of the lower river, *A. campanulatum* at the upper site had an average of 37% infestation in all four seasons. In general, *Apiosoma* did not show measurable fluctuations with seasons.

DISCUSSION

Taxonomy and host-symbiont studies of *Trichodina* and *Apiosoma* infesting fishes in the United States have received surprisingly little attention considering the frequency with which these organisms have been associated with fish diseases (Khan et al. 1974, Cone and Odense 1987, Khan 1991). Wellborn (1967) described 13 species of *Trichodina* in southeastern United States, but few reports have been published for this ciliate west of the Mississippi River (Hechmann et al. 1987). Little information is available on *Apiosoma* studies in this country, which is not the case in the former Soviet Union (Bauer 1984). *Cottus bairdi* represents a new host record for *Trichodina tenuiformis* and *Apiosoma campanulatum*.

Comparative Morphology

At the LM level comparison of the adhesive disc of *T. tenuiformis* with that of other species of *Trichodina* reveals a few similarities. *Trichodina reticulata* Hirschmann and Partsch, 1955 described from *Carassius auratus* has denticles similar to *T. tenuiformis* (Bauer 1984). The adhesive disc of the former has a central light zone separated into reticulated structures. But *T. reticulata* differs in having larger overall dimensions (average adhesive disc diameter is 60 μm vs. 25 μm for our material). *T. tenuiformis* has a close affinity to *T. elegans* described by Stein (1979) from fish in Russia. The latter is characterized by an unbroken light zone in the adhesive disc. Our specimens have various-sized light forms in the center of the adhesive disc. To a lesser extent *T. tenuiformis* is similar to *T. puytoraci* Lom, 1962 and *T. domerguei* Dogel, 1940; however, denticle shape and structure of the adhesive disc clearly distinguish *T. tenuiformis* from these species.

Surface features of the adhesive disc and arrangement of the aboral ciliary complex of *T.*

tenuiformis seen by SEM were generally similar to those described for *T. truttae*, an ectoparasite on pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Oncorhynchus mykiss*; Arthur and Margolis 1984), and *T. labrisomi*, an ectoparasite on hairy blenny (*Labrisomas nuchipinnis*; Rand 1993). However, in *T. tenuiformis*, aboral cilia length is generally shorter than in those previously described. Furthermore, comparison of the aboral ciliature of *T. tenuiformis* with these species of *Trichodina* showed some differences in the extent of development of the anterior and basal septa, in velum structure, and in the degree of evidence of the marginal ciliary ring. The anterior septum is relatively large and the basal one is small in *T. truttae*, whereas in *T. tenuiformis* the basal septum is prominent. The velum is well developed in both *T. labrisomi* and *T. tenuiformis*, but *T. tenuiformis* lacks any protuberances (Rand 1993). Similar to *T. labrisomi*, the marginal ciliary ring of *T. tenuiformis* is poorly developed and cannot be distinguished from the locomotor ciliary ring, whereas in *T. truttae* the marginal ciliary ring is well developed (Arthur and Margolis 1984). Rand (1993) has suggested these marginal ciliature are sensory structures associated with feeding and orientation. Unlike *T. labrisomi* and *T. truttae*, *T. tenuiformis* has no pellicular pores between denticles and the pellicular ridges on the oral surface, which might be a species-specific characteristic for these two species respectively (Arthur and Margolis 1984, Rand 1993).

Over 50 species of *Apiosoma* have been recorded from fishes, the majority of which have been described by Russian authors (Bauer 1984). Although some are common fish parasites in some parts of the world, only one reference concerning *Apiosoma piscicola* on *Salvelinus fontinalis* was reported in North America (Cone and Odense 1987). There is a paucity of data pertaining to *Apiosoma* over the last two decades, likely reflecting taxonomic difficulties due to variability in ciliary structure and lack of strict host-specificity.

Apiosoma conica has a body shape similar to *A. campanulatum*. But our specimens compared more closely to the original description of *A. campanulatum*.

The species identifications were based on original descriptions from Europe; there is a possibility that the two species described in this content are not absolutely identical on both continents.

Host-Symbiont Relationships

Trichodina tenuiformis is an ectocommensal with a tendency to be parasitic in mottled sculpin. There were no visible pathological symptoms with light microscopy; however, electron microscopy disclosed changes in the organelles of host epithelial cells infested by *T. tenuiformis*. Mitochondria decreased in number and disappeared, which might indicate respiratory blockage due to lack of oxygen. This change in mitochondria was observed in *Trichophrya* infesting other fish (Heckmann and Carroll 1985). Necrotic host epithelial tissue sloughs off following organelle loss, supplying sustenance for the parasite.

No serious damage to mottled sculpin could be observed for *A. campanulatum*. Lom (1973) suggested that this simple ectocommensal relationship could change to parasitism in case of heavy invasions, although this tendency is much less pronounced than in trichodinids.

Ecological Aspects of Infestation

This study shows that the infestation of *Trichodina* has both seasonal and regional fluctuations. The higher infestation rate on fish came from the Provo residential area during the spring sampling period. Heavy impact from the local human population may contribute to this infestation. After summer, the number of *T. tenuiformis* gradually reduces with the decrease of water temperature and reaches the highest number the following spring. This may be related to the ciliate life cycle.

Unlike *T. tenuiformis* in this study, *A. campanulatum* maintained a fairly constant infestation on mottled sculpin from the two sites on the Provo River in all four seasons.

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